

09/282879

=> d his

(FILE 'HOME' ENTERED AT 11:41:37 ON 13 DEC 2005)

INDEX 'ADISCTI, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, ANTE, AQUALINE, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOENG, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DISSABS, ...' ENTERED AT 11:42:17 ON 13 DEC 2005  
SEA (IMMOBILI? SPHINGOMYELIN)

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1  FILE AGRICOLA
3  FILE BIOSIS
3  FILE BIOTECHNO
4  FILE CAPLUS
3  FILE EMBASE
1  FILE ESBIOBASE
2  FILE LIFESCI
2  FILE MEDLINE
1  FILE PASCAL
2  FILE SCISEARCH
2  FILE TOXCENTER
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## L1 QUE (IMMOBILI? SPHINGOMYELIN)

— 7 —

FILE 'CAPLUS, BIOSIS, BIOTECHNO, EMBASE, LIFESCI, MEDLINE, SCISEARCH, TOXCENTER, AGRICOLA, ESBIODEBASE, PASCAL' ENTERED AT 11:43:19 ON 13 DEC 2005

L2 24 S L1

L3 6 DUP REM L2 (18 DUPLICATES REMOVED)

=> s 11  
L2 24 L1

=> dup rem 12  
PROCESSING COMPLETED FOR L2  
L3 6 DUP REM L2 (18 DUPLICATES REMOVED)

=> d 13 ibib ab 1-6

L3 ANSWER 1 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 1  
ACCESSION NUMBER: 1995:338905 CAPLUS  
DOCUMENT NUMBER: 122:181511  
TITLE: An improved assay method for the measurement and detection of sphingomyelinase activity  
AUTHOR(S): Taki, Takao; Chatterjee, Subroto  
CORPORATE SOURCE: Sch. Med., Tokyo Med. Dental Univ., Tokyo, 113, Japan  
SOURCE: Analytical Biochemistry (1995), 224(2), 490-3 *January 1995*  
CODEN: ANBCA2; ISSN: 0003-2697

PUBLISHER: Academic  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We have developed an improved assay method to measure sphingomyelinase activity and to detect this enzyme separated on polyacrylamide gels. The assay of sphingomyelinase activity involved immobilizing [N-methyl-14C] sphingomyelin on polyvinylidenuoride (PVDF) membrane, incubation with sphingomyelinase, and the measurement of radioactivity associated with [14C] phosphocholine. The enzyme activity was dependent on the concentration of sphingomyelin, enzyme, pH, and temperature. Thirty minutes of incubation time was optimal for enzyme activity. This enzyme had a bimodal pH optimum, in that optimum enzyme activity was measured at pH 5.4 and 7.4. The detection of sphingomyelinase was pursued by separating the enzyme on a polyacrylamide gel and carrying out the enzyme assay by exposure to [14C] sphingomyelin blotted on a PVDF membrane. The enzyme activity on the PVDF membrane was visualized by autoradiog. A white band (depicting hydrolytic removal of [14C] sphingomyelin from PVDF) was observed. Our method of detecting sphingomyelinase by **immobilizing sphingomyelin** on PVDF membrane may serve as a prototype for assaying various other enzymes in which the hydrolytic product is released into the aqueous phase. Moreover, our method for detecting sphingomyelinase on polyacrylamide gels may be helpful in further studies on the mol. biochem. of this and related phospholipases.

L3 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1982:16439 CAPLUS  
DOCUMENT NUMBER: 96:16439  
TITLE: Enzymic hydrolysis by bacterial phospholipases C and D of immobilized radioactive sphingomyelin and phosphatidylcholine  
AUTHOR(S): Malmqvist, Torsten; Moellby, Roland  
CORPORATE SOURCE: Dep. Bacteriol., Karolinska Inst., Stockholm, S-104 01/60, Swed.  
SOURCE: Acta Pathologica et Microbiologica Scandinavica, Section B: Microbiology (1981), 89B(5), 363-7  
CODEN: APBMDF; ISSN: 0304-131X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB An assay system for phospholipase C with sphingomyelin immobilized on octyl-Sepharose CL-4B as substrate has previously been described. The immobilization procedure was further developed and used with [14C-choline] sphingomyelin and [14C-choline] phosphatidylcholine. These immobilized radioactive phospholipids made the enzymic assays easier to

perform and made it possible to increase the sensitivity. Furthermore, since release of the choline part instead of the phosphate part of the substrate mol. was measured, it was possible to use this assay for phospholipase D as well. The characteristics of phospholipase D from *Corynebacterium ovis* were compared in this test system with those of 3 phospholipases C (from *Clostridium perfringens*, *Bacillus cereus*, and *Staphylococcus aureus*) with respect to hydrolyzing capacities and optimal ion concns.

L3 ANSWER 3 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN DUPLICATE 2  
ACCESSION NUMBER: 1982:16438 CAPLUS  
DOCUMENT NUMBER: 96:16438  
TITLE: Enzymic hydrolysis of **immobilized sphingomyelin** by three bacterial phospholipases C  
AUTHOR(S): Malmqvist, Torsten; Malmqvist, Magnus; Moellby, Roland  
CORPORATE SOURCE: Dep. Bacteriol., Karolinska Inst., Stockholm, S-104 01/60, Swed.  
SOURCE: Acta Pathologica et Microbiologica Scandinavica, Section B: Microbiology (1981), 89B(5), 357-61  
CODEN: APBMDF; ISSN: 0304-131X  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Through hydrophobic interaction, sphingomyelin was adsorbed to agarose beads containing octyl groups by a stepwise dilution procedure. This immobilized lipid was used as a substrate for 3 bacterial phospholipases C (EC 3.1.4.3). The degradation with time of this substrate showed the existence of 2 populations of substrate hydrolyzed at different initial velocities when phospholipases C from *Bacillus cereus* and *Clostridium perfringens* were used. The early fractions could be predigested by the enzymes, a procedure which resulted in linear time-curves. The corresponding early part of the time-curve for phospholipase C from *Staphylococcus aureus* was linear, indicating a comparatively large early fraction of the substrate for this enzyme. The stock gel of the immobilized lipid substrate could be stored for months. It was easily and reproducibly handled as a water suspension. After enzymic hydrolysis the substrate was rapidly separated from enzyme and product by filtration. This assay conveniently avoids the difficulties associated with the use of temporary sonicated suspensions as substrate for bacterial phospholipases C.

L3 ANSWER 4 OF 6 BIOTECHNO COPYRIGHT 2005 Elsevier Science B.V. on STN DUPLICATE  
ACCESSION NUMBER: 1981:13151755 BIOTECHNO  
TITLE: Effects of staphylococcal  $\beta$ -haemolysin on **immobilized sphingomyelin** and on the sheep erythrocyte membrane  
AUTHOR: Malmqvist T.; Mollby R.  
CORPORATE SOURCE: Dep. Bacteriol., Karolinska Inst., Stockholm, Sweden.  
SOURCE: Zentralblatt fur Bakteriologie Mikrobiologie und Hygiene - Abt. 1 Orig. A, (1981), 251/Suppl. 10 (253-259)  
CODEN: ZMMPAO  
DOCUMENT TYPE: Journal; Article  
COUNTRY: Germany, Federal Republic of  
LANGUAGE: English

L3 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2005 ACS on STN  
ACCESSION NUMBER: 1982:16574 CAPLUS  
DOCUMENT NUMBER: 96:16574  
TITLE: Effects of staphylococcal  $\beta$ -hemolysin on **immobilized sphingomyelin** and on the sheep erythrocyte membrane

AUTHOR(S) : Malmqvist, T.; Moellby, R.  
CORPORATE SOURCE: Dep. Bacteriol., Karolinska Inst., Stockholm, Swed.  
SOURCE: Zentralblatt fuer Bakteriologie, Mikrobiologie und  
Hygiene, Abteilung 1, Supplemente (1981),  
10(Staphylococci Staphylococcal Infect.), 253-9  
CODEN: ZBMSDR; ISSN: 0172-5629

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Purified sphingomyelinase C (I) from *Staphylococcus aureus* caused the hydrolysis of sphingomylin immobilized on octyl-Sepharose gel. The rate of hydrolysis linearly increased by increasing the incubation time. Maximum hydrolysis was observed after a 60-min incubation and in the presence of 20-100 mM Mg<sup>2+</sup>. Small amts. of Zn<sup>2+</sup> (1.0 mM) inhibited sphingomyelin hydrolysis. I induced complete hemolysis of sheep erythrocytes at 4°. At 37°, only a very low hemolysis occurred. The amount of hydrolyzed sphingomyelin at 20 min represented .apprx.33% of the sphingomyelin available on the outside of the erythrocyte membrane.

L3 ANSWER 6 OF 6 BIOSIS COPYRIGHT (c) 2005 The Thomson Corporation on STN  
DUPLICATE 4

ACCESSION NUMBER: 1982:10397 BIOSIS  
DOCUMENT NUMBER: PREV198222010397; BR22:10397  
TITLE: SCREENING METHOD FOR BACTERIAL PRODUCTION OF SPHINGOMYELINASE.

AUTHOR(S) : MALMQVIST T [Reprint author]  
CORPORATE SOURCE: DEP BACTERIOL, KAROLINSKA INSTITUTET, S-104 01 STOCKHOLM,  
SWED  
SOURCE: FEMS Microbiology Letters, (1981) Vol. 10, No. 1, pp.  
91-94.  
CODEN: FMLED7. ISSN: 0378-1097.

DOCUMENT TYPE: Article  
FILE SEGMENT: BR  
LANGUAGE: ENGLISH

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L2: Entry 2 of 14

File: PGPB

Mar 24, 2005

DOCUMENT-IDENTIFIER: US 20050064440 A1

TITLE: Methods for identifying risk of melanoma and treatments thereof

Detail Description Paragraph:

[0158] It may be desirable to immobilize a target molecule, an anti-target molecule antibody, and/or test molecules to facilitate separation of target molecule/test molecule complexes from uncomplexed forms, as well as to accommodate automation of the assay. The attachment between a test molecule and/or target molecule and the solid support may be covalent or non-covalent (see, e.g., U.S. Pat. No. 6,022,688 for non-covalent attachments). The solid support may be one or more surfaces of the system, such as one or more surfaces in each well of a microtitre plate, a surface of a silicon wafer, a surface of a bead (see, e.g., Lam, *Nature* 354: 82-84 (1991)) that is optionally linked to another solid support, or a channel in a microfluidic device, for example. Types of solid supports, linker molecules for covalent and non-covalent attachments to solid supports, and methods for immobilizing nucleic acids and other molecules to solid supports are well known (see, e.g., U.S. Pat. Nos. 6,261,776; 5,900,481; 6,133,436; and 6,022,688; and WIPO publication WO 01/18234).

[Previous Doc](#)[Next Doc](#)[Go to Doc#](#)